

NOTE TO THE FILE

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Subject: Aventis's (formerly AgrEvo USA Company) "Liberty Link®" Rice, LLRICE06 and LLRICE62 transformation events.

Keywords:

Rice, Liberty Link® Rice, *bar* gene, Phosphinothricin acetyl transferase gene, PAT gene, Herbicide-Tolerant, Glufosinate-Tolerant, *Streptomyces hygroscopicus*.

Background:

In a submission dated November 30, 1999, Aventis (formerly AgrEvo USA Company) provided summary information to support their safety assessment of the glufosinate-ammonium tolerant rice (also called "Liberty Link®" rice) containing transformation events LLRICE06 and LLRICE62. In a submission dated August 4, 2000, Aventis provided additional information regarding the level of some amino acids in their "Liberty Link®" rice.

Intended effect and food/feed use

According to Aventis, the intended effect of the LLRICE06 or LLRICE62 genetic modification is to confer tolerance to the herbicide glufosinate-ammonium (GA). Rice plants derived from transformation events LLRICE06 and LLRICE62 contain a stably integrated *bar* gene from *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin-N-acetyl transferase (PAT). PAT catalyzes the conversion of L-phosphinothricin, the active ingredient in GA, to an inactive form, thereby conferring resistance to the herbicide. Aventis states that, except for their tolerance to GA, their two new rice varieties are not substantially different from varieties currently on the market. Rice or processed rice are used for both humans and animals food.

Molecular alterations and characterization

Aventis reported that they transformed calli from M202 and Bengal medium-grain rice varieties by direct gene delivery of a chimeric *bar* cassette to produce LLRICE06 and LLRICE62 rice lines, respectively. The chimeric *bar* cassette consists of the coding sequence of the *bar* gene from *S. hygroscopicus* flanked by the constitutive promoter and transcription-termination and polyadenylation sequences of the cauliflower mosaic virus (CaMV) 35S-transcript. The N-terminal codon of the wild type *bar* coding region was substituted for the codon ATG.

According to Aventis, the primary transformation events LLRICE06 and LLRICE62 rice were crossed with commercially available and proprietary rice varieties. In both transformation events, the *bar* gene exhibited Mendelian inheritance and segregated in the genetic offspring as a

single gene locus in the T1 and T2 generations.

Aventis performed Southern hybridization analyses to determine the nature, number, and stability of *bar* gene insertions in the transformed lines LLRICE06 and LLRICE62. Southern hybridization was conducted on digested genomic DNA from transgenic plants homozygous for the integrated DNA, non-transgenic plants, and non-transgenic plants supplemented with an equivalent of one copy of the digested transforming plasmid. According to Aventis, the results showed that one intact copy of the *bar* cassette was integrated into the genome of LLRICE62 transformation event and at least one *bar* cassette into the genome of LLRICE06. A complex integration pattern was observed in LLRICE06. Aventis reported that they could not detect any vector backbone, including *nptII*, sequences in LLRICE06 and LLRICE62 transformation events.

Aventis reported that the banding patterns (“fingerprints”) of the transgene “structure” from T2, T3, and T4 generations of LLRICE06 and from T2 and T3 generations of LLRICE62 were identical across generations, confirming the genetic stability of the transformation events.

Expression of genes in transformation events LLRICE06 and LLRICE62

Aventis performed an enzyme linked immunosorbent assay (ELISA) to measure the level of expression of the phosphinothricin acetyl transferase (PAT) protein (a product of the *bar* gene) in rice grain of transformation events LLRICE06 and LLRICE62. According to Aventis, the PAT protein was expressed at very low levels in transgenic rice grain and processed fractions.

Compositional analysis

1) Antinutrients:

Rice contains a small number of antinutritional factors, which are concentrated in the bran fraction. All of the antinutritional factors, except phytic acid, are subject to heat denaturation. These antinutrients include:

- a) Phytic acid: it chelates calcium, zinc, iron and magnesium in the digestive tract of animals, interfering with absorption and decreasing bioavailability of these nutrients.
- b) Trypsin inhibitor: it is a protease inhibitor that causes depression and pancreatic hypertrophy.
- c) Hemagglutinins (Lectins): they are globulins that agglutinate mammalian red blood cells. Hemagglutinin activity is confined to the germ or primary axis of the rice grain and is below the limit of detection in white rice. Rice bran agglutinating activity is completely destroyed when exposed to 100°C water for two minutes with or without 1% sodium chloride

According to the notifier, the levels of the trypsin inhibitor and lectins in non-transgenic and transgenic LLRICE06 rough rice grain were below the limit of detection. The phytic acid content did not vary significantly between transgenic and non-transgenic varieties and all values were similar to the values reported in the literature.

Aventis also measured the levels of the antinutrients in grain, polished rice, and bran of non-transgenic and transgenic LLRICE62 rough rice. The levels of lectins and trypsin inhibitor in grain and polished rice of transgenic and non-transgenic varieties were below the limit of detection. The levels of lectins in transgenic and non-transgenic rough rice bran were also below the limit of detection. The content of the trypsin inhibitor in transgenic LLRICE62 rough rice bran was lower than the non-transgenic counterpart. The phytic acid levels of transgenic LLRICE62 and non-transgenic rough rice grain, polished rice, and bran were comparable and agreed with the values reported in the literature.

2) Rice allergen (16 kD protein)

The allergenic proteins in rice seeds are reported to be present in the albumin plus globulin fractions of rice endosperm proteins and they fall within the 14- 15 kD size. The 16 kD allergenic protein of rice is a salt- soluble albumin belonging to the α -amylase/trypsin inhibitor gene family.

To ascertain whether any change in either the level or allergenicity of the 16 kD protein had occurred in the transgenic rice versus comparable non-transgenic counterpart rice, the notifier performed the radioallergosorbent (RAST) and RAST inhibition tests. RAST inhibition study involved testing the ability of transgenic (LLRICE06 and LLRICE62) and non-transgenic rice extracts to inhibit the binding of a rice-reactive sera pool to transgenic and non-transgenic allergens. The notifier did not detect any significant differences in the endogenous rice allergen content of the transgenic rice extracts as compared to the non-transgenic rice extracts. The notifier also measured rice allergenic protein (14-16 kD) and total extractable protein in LLRICE06, LLRICE62, and their non-transgenic counterparts using ELISA. There were no significant differences in the content of rice allergenic protein and total extractable protein between the transgenic and the non-transgenic varieties.

3) Osborne fraction

Rice is often used as a dietary substitute for wheat in patients suffering from celiac disease. Celiac disease is an intestinal disease that manifests itself as an atrophy of the mucosae of the small intestine, resulting in malabsorption of food. A group of proteins known as prolamines are responsible for the disease. Prolamines are part of the Osborne fraction. The Osborne fraction is composed of albumin, globulin, glutelin, and prolamin proteins. Rice contains the Osborne fraction but in much lower quantities than does wheat, rye, or barley.

The notifier did not find significant differences in the content of these proteins in brown rice samples of LLRICE06 and LLRICE62 as compared to their respective non-transgenic counterparts.

4) Nutrients:

Rice products for human consumption are produced from whole grains (rough rice, parboiled rice, and milled rice), rice flour and rice starch. The nutritional composition of various rice fractions and related products differ due to relatively high concentration of fat, protein, fiber,

and vitamins in the bran and germ. The primary nutrients provided to humans by rice products are carbohydrate and protein, although rice products containing bran also provide some vitamins, fat, and fiber. White rice is traditionally enriched to restore some of the vitamins and minerals that are lost during the milling process.

Rice products for animal consumption include short grain rice, broken rice, rice straw, rice hulls, rice bran, rice polishings, and rough rice. Short grain rice and broken rice have a major use as pet food. Rough rice has almost no use in animal feed because of its low nutrient value and high cost as compared to other feeds. Rice polishings, a by-product of milling, provide a nutritious feed product.

The intended effect of the genetic modification is to produce rice that is tolerant to glufosinate. Aventis did not anticipate any other effects from the introduction of the *bar* gene into rice. Nonetheless, Aventis conducted extensive compositional analyses of grain, straw, and processed fractions obtained from the non-transgenic and transgenic LLRICE06 and LLRICE62 varieties to confirm that there are no unintended effects of the genetic modification.

a) Grain and straw:

Aventis determined the composition profile of grain and straw of the LLRICE62 and LLRICE06 (sprayed and unsprayed with GA herbicide) and the non-transgenic rice and compared it to the literature values. The level of proximates (moisture, ash, fat, protein, total dietary fiber, and carbohydrates) in grain and straw of transgenic and non-transgenic rice were similar and close to the values reported in the literature. The amino acid and fatty acid profiles of the LLRICE06 and LLRICE62 transgenic rice grain were similar to those of the non-transgenic rice and comparable to the literature values.

According to Aventis, the mean concentration of minerals (calcium, phosphorus, and iron) and niacin in transgenic and non-transgenic rice were similar and within the ranges of standard values. The level of vitamins B1 and B2 in both transgenic and non-transgenic rice exceeded the standard values reported in the literature. Aventis states that these high values may represent variability in the vitamin content of grain since the published standard values also exhibit high variability.

b) Processed fractions of rice:

Aventis performed a large number of analyses on processed fractions from transgenic LLRICE06 (sprayed and unsprayed with GA) and LLRICE62 and from the non-transgenic rice and compared it to the literature values. The processed rice fractions included:

- 1- rice hulls
- 2- brown rice: the remaining kernel following the removal of the hulls, with the bran remaining
- 3- parboiled brown rice: the rough rice that has gone through a steam-pressure process before milling. This process gelatinizes the starch in the grain to ensure firmer, more separate grain.
- 4- polished rice: milled, polished brown rice to remove traces of the bran.
- 5- rice flour: used to produce rice pasta, chips, and other snacks. It is used as a wheat flour

substitute for persons who are allergic to gluten

6- rice bran: gives the brown rice its color and is a rich source of vitamins and minerals. Non-food grades of rice bran are used to feed livestock.

7- bran oil: studies show that bran oil is effective in reducing cholesterol in the blood.

According to Aventis, the levels of proximates, amino acids, fatty acids, minerals and vitamins in processed fractions of LLRICE06 and LLRICE62 transgenic varieties and their respective non-transgenic counterparts were comparable and generally close to the levels reported in the literature.

Feeding studies:

Aventis confirmed the wholesomeness and the nutritional equivalence of the transgenic rice through a feeding study where male broiler chicken were fed for 42 days with either the LibertyLink® transgenic rice varieties or with a related conventional rice variety.

Conclusions

Aventis has concluded that its transgenic Liberty Link® rice containing transformation events LLRICE06 or LLRICE62 are not materially different in terms of food safety and nutritional profile from non-transgenic rice currently on the market. At this time, based on Aventis's description of its data and analysis, the agency considers Aventis's consultation on LLRICE06 or LLRICE62 rice events to be complete.

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